

REMARKS

Upon entry of the present amendments, claims 18-20, 33, 43, 48 and 66-67 will be pending in the application. Claims 18 and 33 have been amended to incorporate the limitations of base claims 1 and 63, and base claims 31 and 26, respectively. Support for the amendment to claim 18 appears at least, *e.g.*, in claims 1, 5 and 63 as filed, and in the specification at least, *e.g.*, at page 26, lines 3-14, at page 27, lines 5-18, in Examples 9 and 10, in Figures 16-18, and in Table 2 on pp. 34-35. Support for the amendment to claim 33 appears at least, *e.g.*, in claims 26 and 31 as filed, and in the specification from page 55, line 12, through page 56, line 7. Claims 43 and 48 are amended to more particularly point out the subject matter of the claims. Support for new claims 66 and 67 appears at least, *e.g.*, in claim 1 as originally filed, and in the specification at least, *e.g.*, at page 12, lines 18-23. The Title and Abstract have been amended to overcome informalities. Formal drawings are being filed herewith. No new matter has been added.

Examiner's Position.

In the Office Action the Examiner made the following objections:

- (1) The Abstract was objected to for containing the word "novel" and for allegedly referring to speculative application of the invention.
- (2) The title was objected to for not being descriptive of the elected invention.
- (3) The Drawings were objected to by the Draftsperson for containing informalities.
- (4) Claims 18 and 33 were objected to as being in improper dependent form for being dependent on a non-elected claim.

In the Office Action the Examiner made the following rejections:

- (1) Claims 18-20, 33, 43 and 48 are rejected under 35 U.S.C. §101 for lack of utility.
- (2) Claims 18-20, 33, 43 and 48 are rejected under 35 U.S.C. §112, ¶ 1, in conjunction with 35 U.S.C. §101, for lack of enablement.
- (3) Claims 18-20, 33, 43 and 48 are rejected under 35 U.S.C. §112, ¶ 1, for lack of written description.
- (4) Claim 33 is rejected under 35 U.S.C. §112, ¶ 1, for lack of written description.

- (5) Claims 43 and 48 are rejected under 35 U.S.C. §112, ¶ 1, for lack of enablement.
 - (6) Claim 33 is rejected under 35 U.S.C. §112, ¶ 2, for indefiniteness.
 - (7) Claims 18-20, 33, 43 and 48 are rejected under 35 U.S.C. §102 for anticipation by Kurokawa *et al.* (U.S. Pat. No. 5,571,895)(“Kurokawa”).
 - (8) Claims 20 and 48 are rejected under 35 U.S.C. §103(a) for obviousness, as being unpatentable over Kurokawa in view of Cruse *et al.* (Illustrated Dict. of Immu. 1995)(“Cruse”).
- Each will be addressed in turn below.

Objections to the Specification are overcome.

The Examiner objected to the Abstract and Title and has noted informalities in the Drawings. The Abstract and Title have been amended. Formal Drawings are being filed concurrently herewith in a separate mailing to BOX Official Draftsperson.

These objections are now moot and should be withdrawn.

Objections to the Claims are overcome.

The Examiner objected to claims 18 and 33 for being improperly dependent on unelected claims. Claims 18 and 33 have been rewritten in independent form, incorporating the subject matter of the base claims and any intermediate claims. These objections are now moot and should be withdrawn.

35 U.S.C. § 101 Utility rejections are overcome, both alone and in combination with the 35 U.S.C. § 112, first paragraph, rejections.

Claims 18-20, 33, 43 and 48 are rejected by this Examiner as lacking utility and as being non-enabled. Specifically, the Examiner contends that because the instant claims are drawn to an antibody that immunospecifically binds a protein characterized by the Examiner as having a yet undetermined function or biological significance, the claimed invention cannot have any substantial, or “real world” utility (Office Action, pp. 5-10). As such, the Examiner asserts that the claims fail to adequately teach how to use the claimed invention (Office Action, p. 10). Applicants traverse for the reasons set forth below.

Specific, substantial and credible utility for the claimed FGF-CX polypeptide (SEQ ID NO:2; *see*, FIG. 1), FGF-CX nucleotide (SEQ ID NO:1; *see*, FIG. 1) and anti FGF-CX antibody is provided throughout the specification. Specific examples of utility include:

- FGF-CX polypeptide has 70-80 % identity and 81-89% positive homology to FGF family members FGF-9 and XFGF-CX. *See, e.g.*, FIGS. 4 - 9.
- FGF-CX residues aa 125-148 have 100% identity to the conserved FGF family domain. *See, e.g.*, page 91, lines 3-7, FIG. 13 double underlined. (alignment repeated below).

Motif:		G.L.G.....EC.F.E.....Y
FGF-CX	125	GELYGSEKLTSECIFREQFEENWY 148

- FGF-CX residues aa 95-120 contain the FGF internal hydrophobic transport domain. *See, e.g.*, page 15 lines 16-24, and Table 1, row 13, on p. 13.
- FGF-CX polypeptide shares the FGF family's functional characteristic of binding heparin sulfate proteoglycan (HSPG) present on the surface of cells and in the extracellular matrix (ECM). *See, e.g.*, Example 7.
- FGF-CX protein demonstrates functional growth factor-like activity *in vitro* in various fibroblast cell lines. *See, e.g.*, Examples 9 and 10, FIG. 16.
- FGF-CX polypeptide demonstrates functional growth factor activity *in vivo*. *See, e.g.*, Example 11.

The FGF-CX polypeptides were initially characterized as members of the FGF family of proteins based on homology. The Utility Examination Guidelines state that "when a patent application claiming a nucleic acid asserts a specific, substantial, and credible utility, and bases the assertion upon homology to existing nucleic acids or proteins having an accepted utility, the asserted utility must be accepted by the Examiner unless the Office has sufficient evidence or sound scientific reasoning to rebut such an assertion." Fed. Reg., Vol. 66. No. 4, January 5, 2001, p. 1096. The FGF-CX polypeptide is fully characterized as a fibroblast growth factor (FGF). The well-established utility for FGF family of proteins include growth, survival, apoptosis, motility and differentiation, as given in Page 9, lines 18-19 of the instant specification. As shown in Examples 9 - 11, Applicants have presented the data showing that the protein of the present invention facilitates cell growth in NIH 3T3 mouse fibroblasts, human cell lines CCD-1070Sk normal human skin fibroblasts and CCD-1106 keratinocytes (Figure 16 in the instant

specification). Furthermore, Applicants present the data wherein NIH 3T3 cells cultured with FGF protein showed 3-fold increase in cell number as compared to the control (Figure 17 in the instant specification), demonstrating a role in cell proliferation. Applicants disclosure of FGF-CX's role in cell growth and proliferation is thus fully supported by experimental evidence, and is a credible, substantial and specific utility. FGF-CX's biological activity is aptly demonstrated and disclosed in the specification.

The Examiner further asserts that the disclosed protein to which the claimed invention immunospecifically binds is incompletely characterized, and that the instant application is therefore directly analogous to *Brenner v. Manson*, 383 U.S. 519 (1966) (Office Action, p.5). However, the Office's reliance on this case is misguided because *Brenner* dealt with a situation in which zero utility was asserted in the application as filed. (*See Brenner*, 383 U.S. at 521-22 and 531-32).

In contrast, the instant Specification makes multiple specific assertions of utility for the claimed invention – in this case, antibodies to the novel fibroblast growth factor-CX (“FGF-CX”) of SEQ ID NO:2. The proteins of this invention may be used to stimulate cell growth, including, for example, growth of fibroblasts and epithelial cells in the linings of the gastrointestinal tract. The Specification expressly states this as a specific, substantial, and credible utility. *See, e.g.*, above, and the following sections in the disclosure:

“The proteins of the invention may be used to stimulate cell growth and cell proliferation in conditions in which such growth would be favorable. An example would be to counteract toxic side effects of chemotherapeutic agents on, for example, hematopoiesis and platelet formation, linings of the gastrointestinal tract, and hair follicles.”

(*See, e.g.*, Specification at p. 78, lines 26-29).

The Specification (pp. 77-79) clearly details stimulation of epithelial cells (including keratinocytes and fibroblasts), glial cells, and cells found in the lining of the gastrointestinal tract. (*See, e.g.*, Specification at p. 77, lines 19-30; and p. 78, lines 1-13). Such stimulation can be used to heal wounds and ulcers. (*See Specification* at p. 77, lines 29-30).

Applicants have provided unequivocal evidence of record that confirms that the proteins in the instant application have precisely this activity. (*See Specification*, FIGS. 16-19, 22, and

26). Additionally, applicants now make of record in the instant application their published work demonstrating that administration of FGF-CX protein “enhances the growth of intestinal fibroblasts”. See Jeffers *et al.*, *Gastroenterology*, 123, pp. 1151-62 (2002) (citing Abstract) (Exhibit 1); *see also*, Proliferation and Neutralization Assays FIG.1 (Exhibit 2).

Moreover, applicants submit herewith a Press Release announcing the FDA approval of CuraGen’s (the assignee of this application) Investigational New Drug application to initiate human clinical trials using FGF-CX to treat oral mucositis. Oral mucositis is a side effect of chemotherapy and radiotherapy that results in degradation of mucosal tissue that can range from redness and irritation to severe ulcerations of the mouth and throat (Exhibit 3). In this trial, the safety and efficacy of FGF-CX’s ability to stimulate cell proliferation (*i.e.*, proliferation of fibroblasts and specifically of epithelial cells) and to counteract toxic side effects of chemotherapeutic and radiotherapeutic agents in the throat and mouth (*i.e.*, linings of the gastrointestinal tract) is being tested. These activities are precisely as recited in the Specification. This meets all requirements to prove utility.

For the record, Applicants note that utility is also supported by the structural similarity of this FGF-CX with other known members of the FGF family and specifically contains a conserved family domain and hydrophobic transport domain. (*See, e.g.*, Specification at p. 14, lines 6-end; p. 15, lines 7-end; and p. 16, lines 3-14). Although the Office seems disinclined to give this fact any weight in its determination of utility, Applicants point out that utility has been recognized for compounds with similar structural features in other cases having facts like the instant application. *See, e.g.*, *In re Jolles*, 628 F.2d 1322 (C.C.P.A. 1980) (finding utility for claimed compounds having close structural relationship to other compounds known to be useful in cancer therapy); and *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995) (stating that although it may be true that minor changes in chemical compounds can radically alter their effects, evidence of success in structurally similar compounds is relevant in determining whether one skilled in the art would believe an asserted utility). As evidenced by Exhibit 1, the FGF-CX protein disclosed in the instant application has a biological activity similar to a structurally related fibroblast growth factor-9 (FGF-9) compound already known and tested in the art for activation and/or proliferation of glial cells and fibroblasts (a subset of which are epithelial cells). Compare Nauro *et al.*, U.S. Patent No. 5,512,460, col. 15, lines 39-47 (1996), with *e.g.*, the instant specification at least at p. 77, line 19, through p. 78, line 13, and with Exhibit 1. Although the Examiner has

pointed out that the disclosed protein ‘only shares approximately 70% amino acid sequence similarity/identity with the most closely related protein of the prior art’ (*see* Office Action at p. 8), Comment 19 of the Utility Examination Guidelines published by the Office state: “ When a class of proteins is defined such that the members share a specific, substantial and credible utility, the reasonable assignment of a new protein to the class of sufficiently conserved proteins would impute the same, specific, substantial, and credible utility to the assigned protein” (*See* 66 Fed. Reg. 4, 1096 (January 5, 2001)). At the time this application was filed, it was well known in the art that FGF-9, a member of the FGF family with well-established utility, only had 30% sequence similarity to other members in the family. Thus, 70% homology is comparatively high for this particular family. Furthermore, as indicated in FIG. 2 of Exhibit 2, the disclosed protein is 100% identical to human FGF-20 (SWISSPROT-ACC:Q9NP95 release date: 16 October, 2001), 95% identical to rat FGF-20 (SPTREMBL-ACC:Q9EST9), 94% identical to mouse FGF-20 (SPTREMBL-ACC:Q9ESL9). FGFs have also been demonstrated to be useful in the stimulation of wound healing. (*See, e.g.*, U.S. Patent Nos. 5,804,213 and 5,728,546). Thus, one utility asserted here for an FGF-CX protein, namely diagnosing and treating cell proliferation associated disorders such as epithelial cell proliferation and wound healing associated with oral mucositis, is supported and consistent with generally accepted scientific principles. One utility is all that is required. *See, In re Gottlieb*, 328 F.2d 1016, 1018 (C.C.P.A. 1964)(holding that one specific utility for a claimed invention is sufficient to meet the utility requirement under § 101).

Neither is the Office’s reliance on Galzie *et al.* (Biochem. Cell Biol. 75: 669-685, 1997) dispositive of the utility requirement in the instant application. It is misleading to assert that none of the associated functions of the nine members of the FGF family are found in common with any other family member. (*See*, Office Action at p.8). For example, it was well known to those of ordinary skill in the art at the time this application was filed that although members of the FGF family have unique functions, each member also has overlapping functions. (*See* Greene *et al.*, U.S. Patent No. 5,728,546, cols. 1-2). Thus, the biological function of the protein family to which the FGF-CX protein is a member is not so diverse as the Office would have the Applicants believe. (*See* Office Action at p. 8). In fact, Table 1 of the Galzie reference, as cited by the Office, provides that several members of the FGF family are generally involved in stimulation of cellular proliferation.

As presented above, the disclosed FGF-CX protein possesses utility under the requirements of § 101, and the invention is fully enabled under § 112, ¶ 1, alone or in combination with § 101. Therefore a rejection based on the hypothesis that an antibody against FGF-CX has no utility if FGF-CX has no utility is not applicable and should be withdrawn.

The presently claimed antibodies of the invention have utility for use in diagnostic applications directed against the FGF-CX protein disclosed in the specification, (*see, e.g.*, Specification, § 11 under heading Anti-FGF-CX Antibodies, p. 54). Disclosed applications include their use as antibody therapeutics, (*see id.*, § 12, p. 55), as pharmaceutical compositions, (*see id.*, § 13, p. 56), and in neutralizing cellular proliferation diseases associated with FGF-CX proteins, such as inhibiting the growth of tumor cells. (*See* Specification, p. 78, lines 9-13).

Furthermore, such utilities would be credible to a person of ordinary skill in the art as they are contemplated in, *e.g.*, Kurokawa, U.S. Patent No. 5,571,815, Example 18, cols. 41-42, a reference cited by the Examiner. Applicants also note for the record that Comment 20 of the Utility Examination Guidelines, published by the Office, indicates that most often, the closest prior art cited and applied in the course of examining the application will demonstrate a well-established utility for the claimed invention. Applicants submit that this is true for the instant application as the claimed antibody of the invention is against a member of the FGF family of proteins that has been shown to have similar functions to those disclosed in U.S. Patent No. 5,571,815.

Finally, to further demonstrate utility for the claimed invention, Applicants submit herewith, in FIG. 3 of Exhibit 2, a Neutralization Assay conclusively showing the inhibition of FGF-CX-stimulated cellular proliferation of NIH 3T3 (fibroblast) cells by anti-FGF-CX IgG, purified from rabbit. FIG. 3 is provided merely to further substantiate statements and assertions already made in the Specification as filed, namely that the claimed invention can be used to neutralize the interaction of FGF-CX proteins for therapeutic or diagnostic purposes. (*See*, Specification at p. 78, lines 9-13, and p. 102, lines 18-19; *See also*, M.P.E.P. 2107 (II)(B)(1)(ii)).

The Examiner has also implicated a “how to use” utility-based 37 C.F.R. § 112, first paragraph, rejection. (*See* Office Action, p. 10). This rejection cannot stand. To uphold a utility-based § 112, first paragraph, rejection, a case must represent one of those rare instances that meets the stringent criterion of being “totally incapable of achieving a useful result.” *Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555 (Fed. Cir. 1992), as discussed

in the Legal Analysis accompanying the Utility Guidelines (M.P.E.P. § 2107). The only instances in which the Federal courts have found a lack of patentable utility were where, “based upon the factual record of the case, it was clear that the invention *could and did not work* as the inventor claimed it did.” M.P.E.P. § 2107 (emphasis added). These rare cases have been ones in which the applicant either (a) failed to disclose any utility for the invention, or (b) asserted a utility that could be true only “if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art.” M.P.E.P. § 2107.01. That is simply not the case here -- as is plain from the Jeffers paper (Exhibit 1), the Proliferation and Neutralization Assays (Exhibit 2), and the FDA’s approval of the IND (Exhibit 3), discussed above.

These rejections should be withdrawn.

35 U.S.C. § 112, first paragraph, rejection is overcome.

Claims 18-20, 33, 43 and 48 were rejected for written description on the ground that the specification does not provide a complete structure to various polypeptide sequences such as variants and fragments of SEQ ID NO:2, and polypeptides having at least 85% sequence identity to SEQ ID NO:2. *See* Office Action, p. 12. Further, the Examiner characterizes the claims as failing to recite other relevant identifying characteristics so as to describe the invention in such full, clear, concise and exact terms that one of ordinary skill in the art would recognize that applicants were in possession of the claimed invention as of the filing date. *Id.* Applicants traverse the rejection as applied to the claims as amended.

First, Applicants acknowledge with appreciation the Examiner’s statement on page 11 of the Office Action that SEQ ID NO:2 meets the written description and enablement requirements.

Second, without acceding to the propriety of the Examiner’s rejection, and in order to expedite the prosecution of this application, Applicants have amended claim 18 so that it no longer recites “a mature form” of a protein, or “a variant of a mature form” of a protein.

The mature FGF-CX is rewritten into new claims 66 and 67, in order to more particularly point out the embodiment where the amino acid sequence of SEQ ID NO:2 comprises a post-translational modifications other than a proteolytic cleavage. Modifications other than proteolytic cleavage are disclosed in the specification as including, by way of non-limiting

example, “myristoylation or phosphorylation.” See page 12, lines 16-19. It would be routine to one skilled in the art at the time of the invention to analyze the elected polypeptide for consensus phosphorylation or myristylation sites because the consensus sites for each were known at the time of filing. An example analysis of the FGF-CX polypeptide using the PROSITE protein domain matching software is provided as Exhibit 4. Consensus sites were located for phosphorylation by protein kinase C, casein kinase II and tyrosine kinase. Additional consensus sites include those for N-myristoylation and amidation. See Exhibit 4. The presence of the FGF domain was also confirmed. Further software programs were available that predict whether or not a polypeptide will undergo cleavage in a cell, and if so, at what position. One skilled in the art at the time of the invention knew these consensus sites and software programs. Therefore, in contrast to the Examiner’s statement in the bridging paragraph between pages 11-12, the mature form of a polypeptide can be reasonably predicted from the amino acid sequence of the entire protein. Accordingly, Applicants believe that these rejections do not apply to the claims as amended and request that such rejections be withdrawn.

With respect to the recitation of variants and fragments of SEQ ID NO:2 in claim 18, Applicants respectfully submit that there is adequate description in the Specification to convey that they contemplated and possessed such various polypeptides at the time of filing. For example, SEQ ID NO:2 (already acknowledged by the Examiner as meeting the written description and enablement requirements) defines and limits the structure of any effective variants or fragments thereof, such that one of ordinary skill in the art would be able to immediately envisage such variants and fragments embraced by the claim. The variants and fragments of SEQ ID NO:2 are further limited by functional characteristics thereby providing further distinguishing characteristics of the claimed invention. (See Synopsis of Application of Written Description Guidelines for the Revised Interim Written Description Guidelines Training Materials, Examples 13 & 15). The above, taken together with the following points (i)-(v), could only lead one ordinarily skilled in the art to conclude that Applicants were in possession of the claimed invention at the time of filing, and therefore, that the claimed invention is adequately described. Points (i)-(v) mentioned above are as follows:

- i) guidance appears in the Specification and claims as to when a variant of SEQ ID NO:2 ceases to be a variant, *i.e.*, when the variant has less than 85% identity to SEQ ID NO:2;
- ii) changes that could be made to produce such variants include, *e.g.*, Table 2, pp. 34-35;

iii) fragments of SEQ ID NO:2 are exemplified and specific range of residues, namely 24-211 and 54-211 of SEQ ID NO:2, are recited at *e.g.*, Specification, p. 3, lines 5-6;

iv) fragments are defined as being biologically active, *e.g.*, Specification, p. 107, Example 17; and

v) a person having a general level of knowledge and skill in the art at the time of the invention would know how to make such variants and fragments.

Contrary to the Examiner's assertion on page 13 of the Office Action, the specific molecular structure on which the variants and fragments are based has been provided, and direction on how to recognize such variants and fragments embraced by the claims has been set forth. To list all the specific molecular structures of variants as contemplated by Table 2 is merely redundant and not required for understanding by one of ordinary skill in the art. More importantly, it is not required in order to adequately meet the written description requirement as set forth under 35 U.S.C. § 112, ¶ 1.

In addition, it is the nature of antibodies that they recognize and bind to specific epitopes. An antibody that recognizes an epitope present in the full length FGF-CX protein will also recognize the same epitope when it is present in a fragment of the FGF-CX protein. Hence, one skilled in the art would know that the scope of the claimed antibody would inherently include an ability to bind its FGF-CX epitope wherever the epitope may occur, whether it be on full length, less-than-full-length, mature or variant forms of FGF-CX.

Since the Specification provides adequate written description of the various polypeptides recited by the claims as amended, the claims drawn to antibodies that immunospecifically bind to such polypeptides are also adequately described. The rejection of these claims should be withdrawn.

Claim 33, which depends from claims 31 and 26, was rejected under 35 U.S.C. §112, ¶ 1, for lack of written description on the ground that it encompasses agents that were not originally contemplated by Applicants (*See* Office Action, p. 14). Applicants have amended claim 33 and submit that it is now commensurate with the scope of the Specification. Accordingly, the rejection should be withdrawn.

Claims 43 and 48 were rejected under 35 U.S.C. §112, ¶ 1, for lack of enablement on the ground that the Specification does not teach how to use a pharmaceutical composition of the claim in an animal without undue experimentation. Applicants note that animal studies are not a necessary prerequisite to patentability of a compound. However, without acceding to the propriety of the Examiner's position, and to expedite prosecution of this application, Applicants have amended the claims as suggested by the Examiner on page 16 of the Office Action. Applicants reserve the right to pursue this subject matter in a later application. As such, this rejection is now moot and should be withdrawn.

The 35 U.S.C. § 112, Second Paragraph Rejections Are Overcome.

Claim 33 was rejected as being indefinite for reciting a "molecular weight not more than 1500 Da." Applicants believe this rejection is now moot in view of the amendment to claim 33 made herein. Accordingly, the rejection should be withdrawn.

The 35 U.S.C. § 102(b) Rejection Is Overcome.

Claims 18-20, 33, 43 and 48 were rejected under 35 U.S.C. §102 for anticipation by Kurokawa. Claims 19-20, 33, 43 and 48 depend from claim 18. Applicants traverse the rejection as applied to the claims as amended.

An alignment of SEQ ID NO:3, as disclosed by Kurokawa, and SEQ ID NO:2 of the present invention indicates a sequence identity of only 70% (*See* Figures 4-6 of the Specification). The variant polypeptide disclosed in claim 18 requires at least 85% identity to SEQ ID NO:2. Furthermore, even if various substitutions were made to SEQ ID NO:2 according to Table 2 of the Specification, the resulting variant polypeptides would still not be anticipated by SEQ ID NO:3 of Kurokawa. Similarly, the size limitation pertaining to the polypeptide fragments of SEQ ID NO:2 obviate the rejection. Since claims 19-20, 33, 43, and 48 each depend, directly or indirectly, from claim 18, the same arguments apply to these claims. Accordingly, the rejection should be withdrawn.

The 35 U.S.C. § 103(a) Rejection Is Overcome.

Claims 20 and 48 are rejected under 35 U.S.C. §103(a) for obviousness, as being unpatentable over Kurokawa in view of Cruse. Applicants traverse the rejection as applied to the claims as amended.

Claims 20 and 48 depend, either directly or indirectly, from claim 18. As discussed under the section regarding § 102, SEQ ID NO:3 disclosed by Kurokawa does not anticipate SEQ ID NO:2 of the instant application, or variants or fragments thereof. Therefore, Kurokawa could not have contemplated the antibody of the present invention, or compositions thereof. Accordingly, even if it were proper to combine the references, the combination does not show every element recited by claims 20 and 48, which depend directly, or indirectly, from claim 18.

In addition, the references lack any suggestion that they be combined in a manner required to meet the claims of the invention. The Cruse reference is from a medical dictionary. It is neither compelling nor persuasive to combine this reference with a patent that is not on point with the present invention, especially when discussing packaging and commercial exploitation of a composition and kit as provided in the recited claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

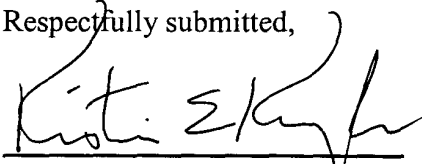
Applicants submit that the application is in condition for allowance, and such action is respectfully requested. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

A petition requesting a two month extension of time and permission to charge the \$205.00 fee under 37 C.F.R. §1.17(a)(2) is enclosed. With the petition, this filing is due on or before Monday, April 21, 2003. Formal Drawings are being filed concurrently under a separate cover letter addressed to the Official Draftsperson, as requested in the Office Action.

Applicants: Jeffers *et al.*
U.S.S.N. 09/817,814
Filed: March 26, 2001

No additional fee is believed due at this time. The Commissioner is hereby authorized to charge payment of any filing fees required in connection with the papers transmitted herewith, or credit any overpayment of same, to Deposit Account No. 50-0311 (Reference No. 15966-557 CIP2).

Respectfully submitted,



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30623

PATENT TRADEMARK OFFICE

Applicants: Jeffers *et al.*
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Filed: March 26, 2001

APPENDIX A:
SUBSTITUTE ABSTRACT

**ANTIBODIES TO FIBROBLAST GROWTH FACTOR-CX
AND THEIR METHODS OF USE**

ABSTRACT

The present invention provides FGF-CX polypeptides and polynucleotides, and antibodies that immunospecifically bind to FGF-CX or any derivative, variant, mutant, or fragment of the FGF-CX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the FGF-CX polypeptide, polynucleotide and antibody are used.

TRA 1454233v2

Applicants: Jeffers *et al.*
U.S.S.N. 09/817,814
Filed: March 26, 2001

Exhibit 1

Jeffers *et al.*, *Gastroenterology*, 123: 1151-62 (2002)

A Novel Human Fibroblast Growth Factor Treats Experimental Intestinal Inflammation

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Background & Aims: We recently identified a novel member of the human fibroblast growth factor (FGF) family of signaling molecules, designated FGF-20. In the present study, we examined the activity of this protein in 2 animal models of acute intestinal inflammation and in mechanistic studies in vitro. **Methods:** In vivo experiments consisted of a murine dextran sulfate sodium (DSS) model of colitis and a rat indomethacin model of small intestinal ulceration/inflammation. Cell growth, restitution, gene expression (cyclooxygenase-2 [COX-2] and intestinal trefoil factor [ITF]), and prostaglandin E₂ (PGE₂) levels were examined in vitro. **Results:** In the DSS-colitis model, prophylactic administration of FGF-20 significantly reduced the severity and extent of mucosal damage as indicated by a 55%–93% reduction in luminal blood loss, distal colonic edema, histologic inflammation, and epithelial cell loss relative to animals administered vehicle control. No toxicity was noted during administration of FGF-20 to normal controls. In addition, therapeutic administration of FGF-20 enhanced survival in this model. In the indomethacin–small bowel ulceration/inflammation model, administration of FGF-20 reduced small intestinal weight gain, necrosis, inflammation, and weight loss (36%–53% relative to vehicle control). In vitro studies demonstrated that FGF-20 stimulates growth, restitution, mRNA expression of COX-2 and ITF, and PGE₂ levels in human intestinal epithelial cells and enhances the growth of human intestinal fibroblasts. **Conclusions:** FGF-20, having demonstrated therapeutic activity in 2 experimental models of intestinal inflammation, represents a promising new candidate for the treatment of human inflammatory bowel disease.

Inflammatory bowel disease (IBD) comprises a spectrum of immune-mediated chronic gastrointestinal disorders, including ulcerative colitis and Crohn's disease.^{1–3} In IBD, the integrity of the intestinal mucosa is compromised, and healing of the surface epithelium is accomplished via epithelial cell migration ("restitution"),

proliferation, and differentiation.⁴ Stimulated fibroblasts are believed to play a role in the healing process.⁴

Although many treatments for IBD exist, additional therapeutic approaches are needed because many patients either do not respond to current options or develop significant side effects to medications, thereby precluding their continued use. Because of the inadequacy of current therapies, some IBD patients with refractory disease undergo surgery to remove a portion of the intestine. A new agent that has fewer side effects than current approaches, has sustainable efficacy in patients unresponsive to available drugs, targets therapeutic mechanisms distinct from current medications, and eliminates the need for surgery would offer clinical and pharmacoeconomic benefits. Because the integrity of the intestinal mucosa is breached in IBD, thereby potentiating the uptake of injurious luminal bacterial antigens and cell wall polymers,¹ it follows that agents that facilitate or accelerate epithelial repair may be therapeutically useful. No currently marketed products fall into this category.

One relevant class of agents for the repair of intestinal epithelium is that of the peptide growth factors, including, among others, members of the fibroblast growth factor (FGF) family.^{5,6} FGF receptors are present on intestinal epithelium,^{7,8} and enhanced expression of various FGF family members has been demonstrated in the intestines of IBD patients,^{9–12} suggesting a potential endogenous reparative role for this family of growth factors after injury caused by inflammatory processes.

Abbreviations used in this paper: COX-2, cyclooxygenase-2; DMEM, Dulbecco's modified Eagle medium; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; FGF, fibroblast growth factor; IBD, inflammatory bowel disease; ITF, intestinal trefoil factor; PBS, phosphate-buffered saline; PGE₂, prostaglandin E₂; RT-PCR, reverse-transcription polymerase chain reaction.

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Support for this theory comes from in vitro and in vivo studies demonstrating that FGFs enhance the restitution and proliferation of intestinal epithelium.^{7,13-15} Assuming that naturally occurring reparative factors such as FGFs are present in suboptimal quantities in the intestines of IBD patients, it follows that supplementation with these factors may promote healing and thus alleviate the symptoms associated with IBD. FGFs have in fact shown promise in animal models of IBD.^{13,16-18}

We recently identified and characterized a novel member of the human FGF family that we designate FGF-20.¹⁹ This factor interacts with multiple FGF receptors and displays mitogenic activity on fibroblasts and epithelial cells. In the present study, we examined the in vivo effects of FGF-20 in 2 rodent models of IBD: dextran sulfate sodium (DSS) treatment of mice to induce an ulcerative colitis-like syndrome and indomethacin treatment of rats to induce ulceration and inflammation of the small bowel, as is seen in Crohn's disease. We also performed in vitro studies to explore the mechanisms of action of FGF-20.

Materials and Methods

Purification of FGF-20

The human FGF-20 cDNA¹⁹ was cloned into pETMY, a modified pRSET vector (Invitrogen, San Diego, CA). The resulting construct encodes amino acids 2–211 of FGF-20 preceded by the sequence MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDRWGS, which contains a histidine tag used for purification purposes, as well as additional vector-encoded residues. The vector was transformed into *Escherichia coli* strain BL21 (Novagen, Madison, WI), which was grown to an optical density of 0.6 and infected with CE6 bacteriophage lambda (Novagen) at a multiplicity of infection of 5. The infected bacterial culture was further incubated for 3 hours at 27°C, obtained by centrifugation (4000×g for 15 minutes at 4°C), resuspended in phosphate-buffered saline (PBS) + 0.5 mol/L NaCl + 1.0 mol/L L-arginine, and disrupted with 2 passes through a microfluidizer at 8000 pounds per square inch. Cell debris was removed by centrifugation (10,000 × g for 25 minutes at 4°C) and discarded. The resulting supernatant containing the FGF-20 protein was clarified by filtration through a 0.22-μm low protein-binding filter and loaded onto a column containing nickel-charged Sepharose (Pharmacia Biotech, Piscataway, NJ). The column was washed with PBS + 0.5 mol/L NaCl + 1.0 mol/L L-arginine, and bound protein was eluted with a linear gradient of 0–0.5 mol/L imidazole. Fractions containing FGF-20 were pooled, dialyzed at 4°C against PBS + 1.0 mol/L L-arginine, and loaded onto a column containing uncharged Sepharose (Pharmacia Biotech). FGF-20 was captured in the flowthrough, dialyzed at 4°C against PBS + 1.0 mol/L L-arginine, and sterilized by passage through a 0.22-μm low protein-binding filter. Purified FGF-20 had an

endotoxin level of ≤25 endotoxin units/mg as determined by the Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, MD).

Bromo-Deoxy-Uridine Assay

NIH 3T3 murine embryonic fibroblasts and Balb/MK murine keratinocytes were cultured in 96-well plates to approximately 100% confluence in growth media (NIH 3T3 cells: Dulbecco's modified Eagle medium [DMEM] + 10% bovine calf serum [Invitrogen]; Balb/MK cells: keratinocyte-SFM [Invitrogen]). Before adding FGF-20, Balb/MK cells were prestarved for 24 hours in basal keratinocyte media. FGF-20 was added to cells for 18 hours in the appropriate basal media supplemented with 0.1% bovine serum albumin, and the bromo-deoxy-uridine assay was performed according to the manufacturer's specifications (Roche Molecular Biochemicals, Indianapolis, IN) using a 3 hour bromo-deoxy-uridine incorporation time.

Animals

Mice. Six- to 8-week-old female Balb/c mice weighing 20–22 g were obtained from Harlan Labs (Indianapolis, IN) for use in the DSS model. Between 3 and 5 animals were housed per cage in polycarbonate cages with filter tops and given mouse chow (Harlan Teklab, Madison, WI) and tap water ad libitum. Animals were acclimated for 6 days before experimental use and were sacrificed by CO₂ inhalation at the end of the study.

Rats. Female Lewis rats weighing 175–200 g were obtained from Harlan Labs for use in the indomethacin model. Four animals were housed per cage and given Harlan Teklab rat chow and tap water ad libitum. Animals were acclimated for 8 days before experimental use. At the end of the study, animals were anesthetized with isoflurane and sacrificed by cervical dislocation after blood collection.

Murine DSS Model

DSS (Spectrum Chemicals, Gardena, CA) working solutions were freshly made every other day in tap water and stored at 4°C. FGF-20 was diluted in PBS + 1.0 mol/L L-arginine, and the vehicle solution consisted of PBS + 1.0 mol/L L-arginine. Intraperitoneal (IP) and subcutaneous (SC) injections were both performed in volumes of 10 mL/kg using FGF-20 stocks of the appropriate concentrations so as to achieve the desired final concentration of FGF-20 (5, 1, or 0.2 mg/kg, as indicated in the Results section and in the Figures). At necropsy, the colon was removed, and colon blood content was scored according to the following criteria: 0, normal to semisolid stool, no blood; 1, normal to semisolid stool, blood-tinged; 2, semisolid to fluid stool with definite evidence of blood; 3, bloody fluid. For histopathologic examination, 3 distal colonic regions spaced approximately 1 cm apart were collected into 10% neutral buffered formalin, processed for paraffin embedding, sectioned, and stained with hematoxylin and eosin. Each section was scored for various parameters, and the mean of the scores for each of the regions was determined.

Submucosal edema was quantitated by measuring the distance from the muscularis mucosa to the internal border of the outer muscle layer. Inflammation (foamy macrophage, lymphocyte, and polymorphonuclear cell infiltrate) was assigned a severity score according to the following criteria: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; 5, severe. Glandular epithelial loss and surface epithelial loss were scored using the following criteria: 0, normal; 1, 1%–10% of the mucosa affected; 2, 11%–25% of the mucosa affected; 3, 26%–50% of the mucosa affected; 4, 51%–75% of the mucosa affected; 5, 76%–100% of the mucosa affected. The 3 important scored parameters (inflammation, glandular epithelial loss, and surface epithelial loss) were combined to arrive at an overall histopathology score that indicates the overall damage and could have a maximum score of 15. For each animal, 3 distal colonic regions spaced approximately 1 cm apart were scored, and the means of the scores for each of the regions were determined.

Rat Indomethacin Model

To induce disease, indomethacin (Sigma, St. Louis, MO) was prepared in 5% sodium bicarbonate to 7.5 mg/mL and injected SC into rats on 2 consecutive days in a volume of 1 mL/kg so as to achieve the desired final concentration of 7.5 mg/kg/dose.¹³ FGF-20 was diluted in PBS + 1.0 mol/L L-arginine, and the vehicle solution consisted of PBS + 1.0 mol/L L-arginine + 5 mg/mL bovine serum albumin. Intravenous (IV) tail vein injections were performed in a volume of 1 mL/kg using FGF-20 stocks of the appropriate concentrations so as to achieve the desired final concentration of FGF-20 (5, 1, or 0.2 mg/kg as indicated in the Results section and in the Figures). At necropsy, a 10-cm section of the distal jejunum in the area at risk for lesions was removed and weighed. This jejunum fragment was then used to obtain 5 approximately equally spaced sections that were collected into 10% neutral buffered formalin, processed for paraffin embedding, sectioned, and stained with hematoxylin and eosin for histopathologic examination. Necrosis was scored according to the following criteria: 0, normal; 1, 1%–10% mucosal necrosis; 2, 11%–25% mucosal necrosis; 3, 26%–50% mucosal necrosis; 4, 51%–75% mucosal necrosis; 5, 76%–100% mucosal necrosis. Inflammation was scored according to the following criteria: 0, none; 1, minimal inflammation in mesentery and muscle or lesion; 2, mild inflammation in mesentery and muscle or lesion; 3, moderate inflammation in mesentery and muscle or lesion; 4, marked inflammation in the lesion; 5, severe inflammation in the lesion.

Growth Assay

CCD-18Co normal human colonic fibroblasts and FHs 74 Int normal human small intestinal epithelial cells were plated in 6-well plates to approximately 25% confluence in growth media and allowed to attach overnight (CCD-18Co cells: DMEM + 10% FBS [Invitrogen]; FHs 74 Int cells: DMEM + 10% FBS + nonessential amino acids [Invitrogen] + sodium pyruvate [Invitrogen] + 1 mmol oxalacetic acid

[Sigma] + 0.2 U/mL insulin [Invitrogen]). The next day, the growth media was removed and replaced with a 1:1 mixture of DMEM (without or with FGF-20)/growth media. The cells were fed with fresh factor after 3 days and counted after 6 days.

Wounded Monolayer Repair Assay

An *in vitro* healing assay was performed using a modified version of a published method.^{13,20} Briefly, reference lines were drawn horizontally across the outer bottom of 24-well plates. HT-29 and Caco-2 human colon carcinoma cells were plated and grown to confluence in DMEM + 5% FBS, and then incubated for 24 hours in DMEM + 0.1% FBS. Linear "wounds" were made with a sterile plastic pipette tip perpendicular to the lines on the bottom of the wells. Then 10% FBS (positive control) or FGF-20 was added, and the size of the wound was measured microscopically at various times at predetermined locations corresponding to the reference lines.

Determination of Cyclooxygenase-2 and Intestinal Trefoil Factor Gene Expression by Reverse-Transcription Polymerase Chain Reaction

Cells (HT-29 and Caco-2) were plated and grown to confluence in DMEM + 5% FBS, and then incubated for 24 hours in DMEM + 0.1% FBS. FGF-20 (100 ng/mL) was then added, and total RNA was obtained from the cells after various times using Trizol (Invitrogen) according to the manufacturer's instructions. RNA was reverse-transcribed using 2 µg of total RNA, 15 U of RNA inhibitor, first-strand synthesis buffer (Invitrogen), 5 mmol deoxynucleoside triphosphate (Pharmacia, Upsala, Sweden), 125 pmol random hexamer primers (Pharmacia), and 125 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen) in a final volume of 25 µL. The reaction was performed for 1 hour at 39°C, followed by 7 minutes at 93°C and 1 minute at 24°C, and then slowly cooled to 4°C for 20 minutes. Reverse transcription-polymerase chain reaction (RT-PCR) was performed in a volume of 50 µL containing 5 µL of reverse transcriptase mixture, 1× Taq buffer, 5 pmol of each primer, 2.5 mmol deoxynucleoside triphosphate, and 1 unit of Taq polymerase. The primers used to amplify human cyclooxygenase-2 (COX-2), intestinal trefoil factor (ITF), and β-actin were as follows:

COX-2: sense, 5'-AGATCATCTCTGCGCTGAGTATCTT-3'; antisense, 5'-TTCAAATGAGATTGTGGGAAATTGCT-3'

ITF: sense, 5'-GTGCCAGCCAAGGACAG-3'; antisense, 5'-CGTTAAGACATCAGCCTCCAG-3'

β-actin: sense, 5'-CCAACCGCAAGAAGATGA-3'; antisense, 5'-GATCTTCATGAGGTAGTCAGT-3'

RT-PCR was carried out in a Perkin-Elmer 9600 cycler (Perkin-Elmer, Wellesley, MA) programmed for 20–40 cycles to assess the linearity of the amplification. The PCR products were separated on 2% Tris-acetate/EDTA agarose gels containing gel star fluorescent dye (FMC, Philadelphia, PA). A negative from the gels was taken with an AlphaImager 2000 (Alpha Innotech, San Leandro, CA).

Determination of Prostaglandin E₂ Levels by Enzyme-Linked Immunosorbent Assay

Cells (HT-29 and Caco-2) were plated and grown to confluence in DMEM + 5% FBS, and then incubated for 24 hours in DMEM + 0.1% FBS. FGF-20 was then added, and 24 hours later the culture medium was harvested and assessed for prostaglandin E₂ (PGE₂) levels via enzyme-linked immunosorbent assay (ELISA; Assay Designs, Ann Arbor, MI).

Results

Expression, Purification, and In Vitro Activity of Recombinant FGF-20

Purified human FGF-20 was isolated from *Escherichia coli* engineered to express full-length FGF-20 protein (Figure 1A). The recombinant protein, which contains vector-encoded sequences and a histidine tag at the N-terminus, had a molecular weight of approximately 29 kilodaltons (Figure 1A, Lane 2), close to its predicted molecular weight of 27,739 daltons. Purified FGF-20 was biologically active, as demonstrated by its ability to induce DNA synthesis in murine fibroblasts (NIH 3T3) and epithelial cells (Balb/MK) at half maximal concentrations of approximately 5 ng/mL (Figure 1B). Similar biological activity was obtained with purified recombinant full-length FGF-20 devoid of vector-encoded sequences (data not shown).

Prophylactic Administration of FGF-20 Is Active in a DSS-Mediated Murine Model of Colitis

The effect of FGF-20 on colitis was initially examined in a murine DSS-mediated disease model.^{21,22} In this model, Balb/c mice exposed to DSS for 7 days developed distal colonic inflammation and edema in association with crypt and colonic glandular epithelial loss, erosion, and ulceration, leading to hemorrhage. In this study, DSS-associated effects on the proximal colon were much less severe than on the distal colon and thus are not reported. FGF-20 (5 mg/kg) administered daily via IP injections on each of the 7 days of DSS exposure significantly reduced the extent and severity of mucosal damage (Figure 2). Specifically, FGF-20 resulted in the following protective effects on the distal colon: 93% reduction of blood content scores, reflecting hemorrhagic diarrhea; 76% reduction in submucosal edema; 55% reduction in mucosal inflammation; 57% reduction in glandular epithelial loss; and 84% reduction in surface epithelial loss. FGF-20 administration also inhibited the DSS-induced decrease in colon length. Histopathology sum scores that take into consideration the parameters of inflammation, glandular epithelial loss, and erosion in-

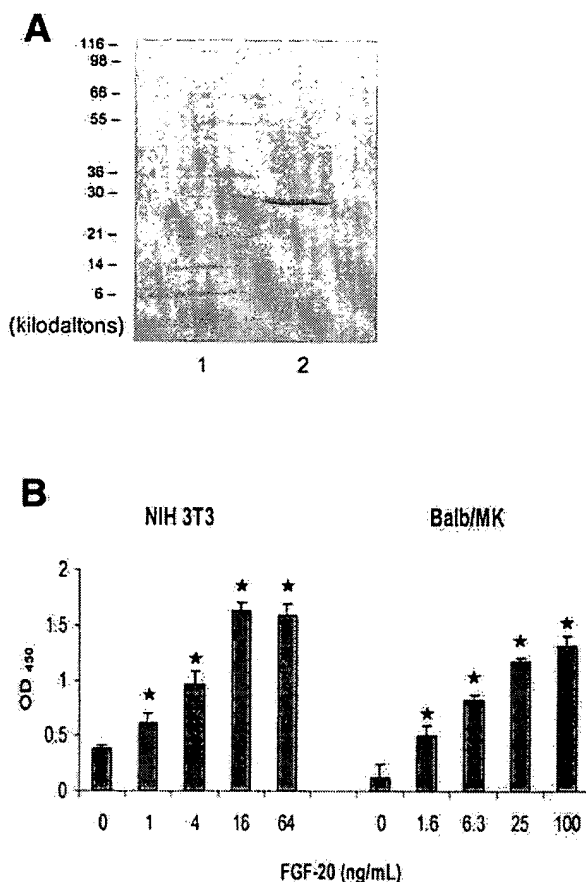


Figure 1. Purification and in vitro biological activity of FGF-20. (A) Full-length FGF-20 protein possessing an N-terminal histidine tag was expressed in *Escherichia coli* and purified to near homogeneity by nickel chromatography. Five micrograms of FGF-20 was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (4%–20% gel) under reducing conditions and visualized with Coomassie stain (lane 2). Lane 1 depicts protein standards (in kilodaltons). (B) NIH 3T3 murine fibroblasts and Balb/MK murine keratinocytes were incubated with purified FGF-20 at the indicated concentrations for 18 hours and analyzed by a bromo-deoxy-uridine incorporation assay. Data points represent the mean of triplicate wells \pm standard deviation. Changes that are statistically different from control cells receiving no factor ($P < 0.05$ by the 2-tailed Student *t* test) are indicated with a star.

dicate that FGF-20 caused a 66% reduction in DSS-mediated effects on the distal colon. Finally, FGF-20 administration reduced the amount of DSS-induced weight loss by 30%. A representative histopathologic example of the protective effect of FGF-20 on the distal colon is depicted in Figure 3, which shows that FGF-20 inhibited the mucosal changes and submucosal edema associated with DSS treatment.

In a follow-up murine DSS-colitis study, we sought to verify the initial results and to determine optimal dosing in the prophylactic protocol using a SC delivery method. Mice were exposed to DSS for 7 days, and FGF-20 (5, 1,

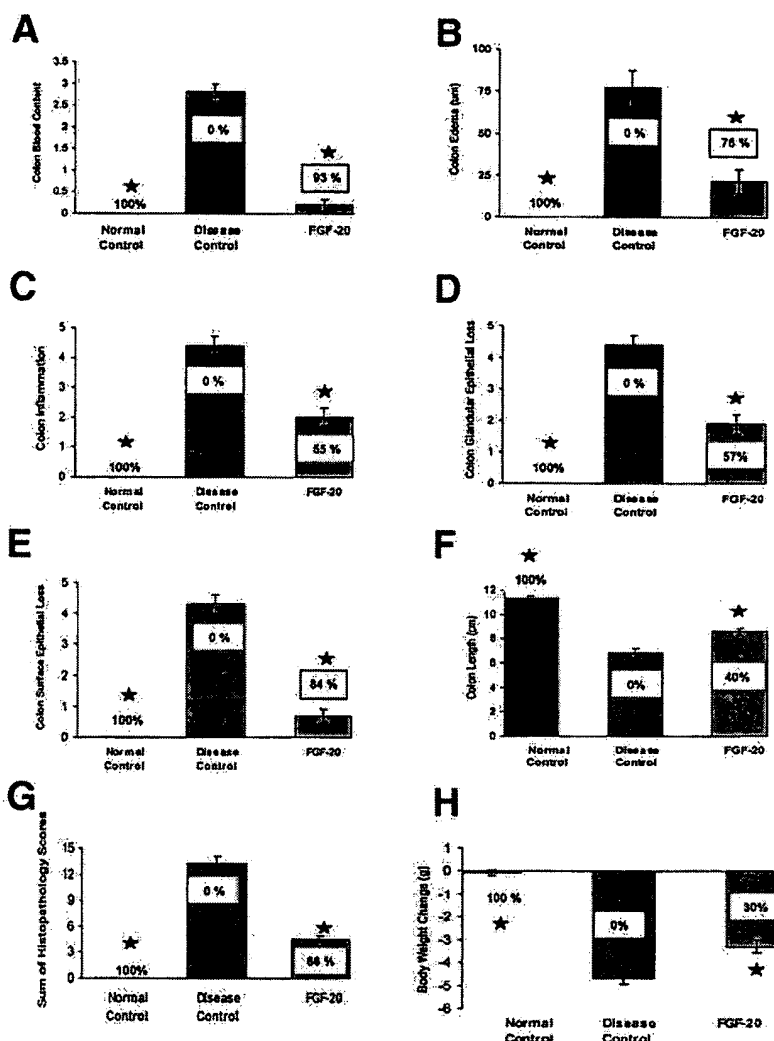


Figure 2. Effects of FGF-20 on DSS-induced colitis. To induce colitis, female Balb/c mice were exposed to 5% DSS in drinking water for 7 days (day 0 to 6). Disease control animals ($n = 10$) received daily IP injections of vehicle solution on each day of DSS exposure. The FGF-20 group of animals ($n = 10$) received daily IP injections of FGF-20 (5 mg/kg) on each day of DSS exposure. Normal control animals ($n = 5$) were not exposed to DSS, but did receive daily IP injections of vehicle solution on day 0 to 6. Animals were sacrificed on day 7, and the distal colon was scored for the following parameters (see Materials and Methods for details): (A) blood content, (B) submucosal edema, (C) mucosal inflammation, (D) glandular epithelial loss, (E) surface epithelial loss (erosion), and (F) length. (G) An overall histopathology score that takes into consideration inflammation, glandular epithelial loss, and erosion in the distal colon. (H) The change in total body weight from day 0 to 7. Results are reported as mean \pm standard error, and the percentage changes indicated are relative to the disease control group. Changes that are statistically different from the disease control group ($P < 0.05$ by the 2-tailed Student t test) are indicated with a star.

and 0.2 mg/kg) was administered daily via SC injections on each of the 7 days of DSS exposure (Figure 4). As was seen in the initial study, FGF-20 treatment reduced the extent and severity of mucosal damage as measured by fecal blood, histologic injury, and colon length, and did so in a dose-dependent fashion with maximum protection offered at the highest FGF-20 concentration examined (5 mg/kg). No significant protective effect of FGF-20 on DSS-induced weight loss was observed in this follow-up study. Significant protection from mucosal

damage was also seen with FGF-20 at 1 mg/kg, whereas 0.2 mg/kg of this factor provided little protection from DSS-induced colitis.

Similar protective effects were obtained with purified recombinant full-length FGF-20 devoid of vector-encoded sequences. Moreover, following the administration of this FGF-20 protein (5 mg/kg SC once daily for 7 days) to normal nondisease control animals, an analysis of animal weight, blood hematology/clinical chemistry, and histopathology on 28 different tissues

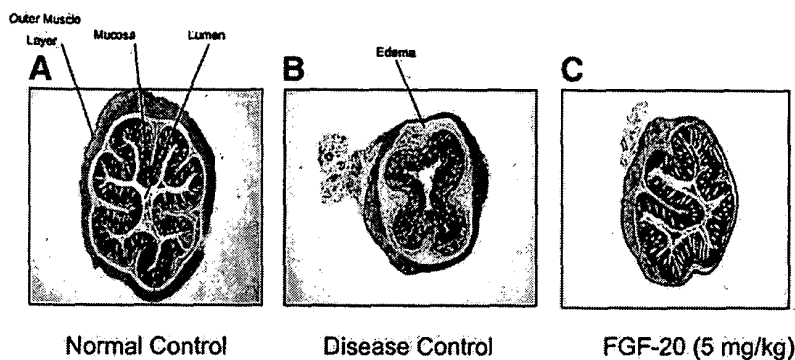


Figure 3. Effects of FGF-20 on DSS-induced colitis: histopathology. Representative sections of distal colon were collected at necropsy, preserved in formalin, stained with H&E, magnified 50 \times , and photographed. The groups are as described in the legend to Figure 2. Note that FGF-20 (5 mg/kg) inhibits the mucosal changes and submucosal edema associated with DSS treatment.

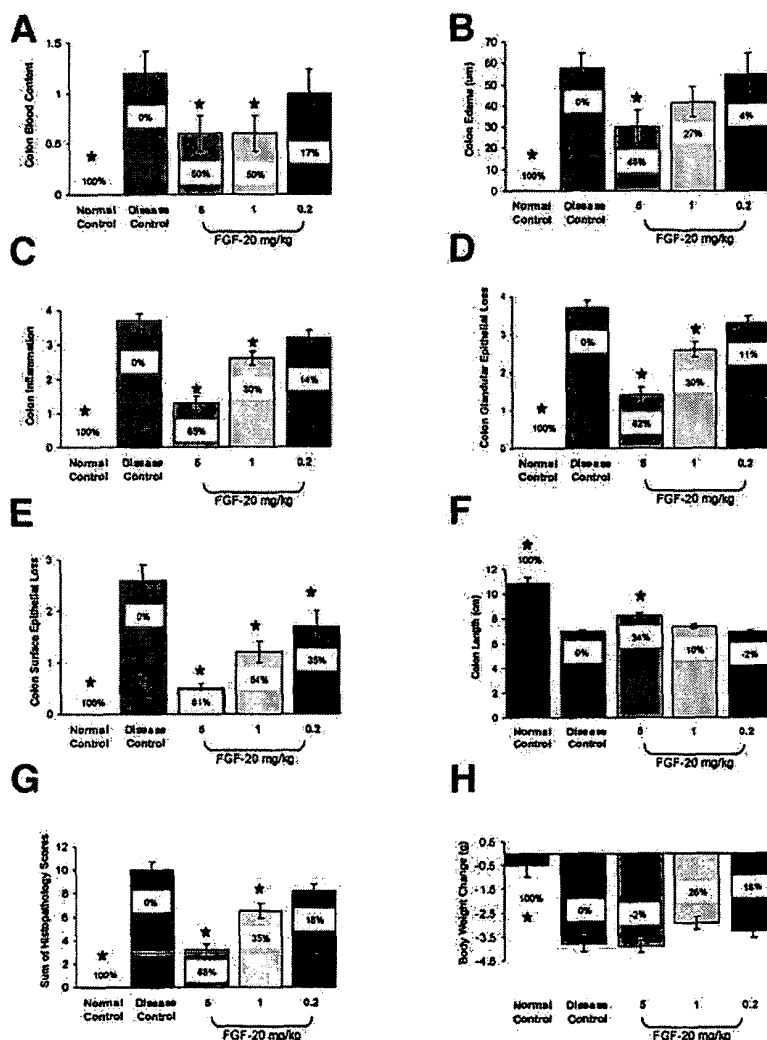


Figure 4. Effects of FGF-20 on DSS-induced colitis: dose response. To induce colitis, female Balb/c mice were exposed to 4% DSS in drinking water for 7 days (day 0 to day 6). Disease control animals ($n = 9$) received daily SC injections of vehicle solution on each day of DSS exposure. The FGF-20 group of animals ($n = 9$) received daily SC injections of FGF-20 at the indicated concentrations on each day of DSS exposure. Normal control animals ($n = 4$) were not exposed to DSS, but did receive daily SC injections of vehicle solution on day 0 to day 6. Animals were sacrificed on day 7, and the distal colon was scored for the following parameters (see Materials and Methods for details): (A) blood content, (B) submucosal edema, (C) mucosal inflammation, (D) glandular epithelial loss, (E) surface epithelial loss (erosion), and (F) length. (G) An overall histopathology score that takes into consideration inflammation, glandular epithelial loss, and erosion in the distal colon. (H) The change in total body weight from day 0 to 7. Results are reported as mean \pm standard error, and the percentage changes indicated are relative to the disease control group. Changes that are statistically different from the disease control group ($P < 0.05$ by the 2-tailed Student t test) are indicated with a star.

revealed that the only gross tissue alteration induced by FGF-20 was increased injection site inflammation/fibroplasia. This analysis also indicated that FGF-20 induced a moderate increase in absolute neutrophils and cholesterol. (Mean absolute neutrophil counts in FGF-20-treated, vehicle control and nontreated control animals were 1405, 968, and 1130, respectively; *t* test *P* value = 0.030 for comparison of FGF-20-treated to vehicle control. Mean cholesterol counts in FGF-20-treated, vehicle control and nontreated control animals were 100, 79.2, and 77.2, respectively; *t* test *P* value = 0.024 for comparison of FGF-20-treated to vehicle control). The biological relevance of these findings remains to be determined.

Therapeutic Administration of FGF-20 Enhances Survival in the Murine DSS Model

In the experiments described previously, DSS exposure and FGF-20 administration were initiated simultaneously on day 0. In another experiment, the effect of FGF-20 administered after the initiation of DSS treatment was examined. To this end, Balb/c mice exposed to DSS for 7 days (day 0 to day 6) were injected daily SC with various concentrations of FGF-20 (5, 1, and 0.2 mg/kg) beginning on the fifth day of DSS exposure (i.e., day 4) and ending 3 days after the termination of DSS exposure (i.e., day 9). Animal survival was recorded on a daily basis, and the experiment was concluded on day 10. As shown in Figure 5, therapeutic administration of FGF-20 at 5 mg/kg enhanced survival relative to the disease control group. Thus, whereas only 44% (4 of 9) of the animals in the disease control group survived until the end of the study, 89% (8 of 9) of the animals treated with FGF-20 at 5 mg/kg survived. FGF-20 administered therapeutically at lower doses (1 and 0.2 mg/kg) had little or no effect on survival.

FGF-20 Is Active in an Indomethacin-Mediated Rat Model of Small Bowel Ulceration/Inflammation

Treatment of susceptible Lewis rats with indomethacin results in chronic small intestinal linear ulcerations bearing some similarity to those observed in Crohn's disease. This model was used to examine the ability of FGF-20 to treat discrete mucosal ulcers. To this end, Lewis rats treated with indomethacin (7.5 mg/kg SQ) for 2 days (day 0 to day 1) were injected daily IV with various concentrations of FGF-20 (5, 1, 0.2 mg/kg) beginning on the day before the initiation of indomethacin treatment (i.e., day -1) and ending 3 days after the termination of indomethacin treatment (i.e.,

day 4). Animals were sacrificed and examined on day 5. Administration of FGF-20 at 0.2 mg/kg resulted in the following protective effects relative to vehicle-treated disease control animals: 52% reduction in indomethacin-induced small intestine weight increase (as measured from a 10-cm section of distal jejunum taken from the area at risk), 53% reduction in histopathologic intestinal necrosis, and a 38% reduction in histopathologic intestinal inflammation (Figure 6). This concentration of FGF-20 also significantly reduced the indomethacin-induced increase in blood neutrophils by 39% and inhibited weight loss by 36%. Higher concentrations of FGF-20 (i.e., 1 and 5 mg/kg) were less active in this model than the 0.2 mg/kg dose. A representative histopathologic example of the protective effect of FGF-20 on the small intestine is depicted in Figure 7, which shows that FGF-20 inhibits the mucosal ulceration and necrosis normally associated with indomethacin treatment.

FGF-20 Enhances the Growth and Restitution of Intestinal Cells In Vitro

Because the healing of the surface mucosa involves epithelial restitution, as well as epithelial and fibroblast growth and/or activation, we examined the effect of FGF-20 on these processes in vitro. Our findings demonstrate that FGF-20 significantly enhances the growth of normal human colonic fibroblasts (CCD-18Co) and normal human intestinal epithelial cells (FHs Int 74) (Figure 8). This effect of FGF-20 was dose-dependent and resulted in a 2- to 3-fold increase in cell number over the course of 6 days of culture.

To explore the effects of FGF-20 on restitution, a wounded monolayer repair assay was performed on 2 human colonic epithelial cancer cell lines, HT-29 and Caco-2. The results of this assay demonstrate that FGF-20 significantly stimulates wound closure in a concentration-dependent manner (Figure 9). The highest FGF-20 dose examined (100 ng/mL) stimulated closure to a similar degree as that of the positive control (10% FBS). Consistent with these results, FGF-20 was also found to enhance the migration of FHs Int 74 normal human intestinal epithelial cells in a dose-dependent fashion when examined in a modified Boyden chamber assay (data not shown).

FGF-20 Stimulates COX-2 and ITF Expression and PGE₂ Levels in Colonic Cells In Vitro

We next examined the effect of FGF-20 on the mRNA expression of COX-2 and ITF, 2 genes whose

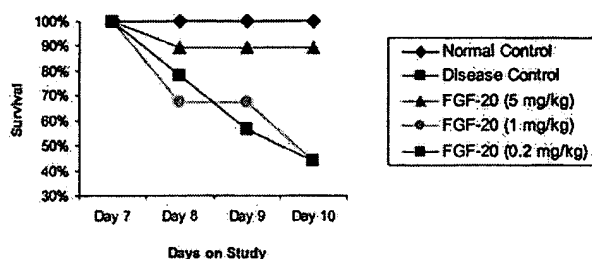


Figure 5. Effect of therapeutically administered FGF-20 on survival in the DSS model of colitis. Female Balb/c mice were exposed to 4% DSS in drinking water for 7 days (day 0 to 6) and then switched to normal drinking water for 4 additional days (day 7 to 10). Disease control animals ($n = 9$) received daily SC injections of vehicle solution on day 4 to day 9. FGF-20 groups ($n = 9$) received daily SC injections of the indicated concentrations of FGF-20 on day 4 to 9. Normal control animals ($n = 3$) were not exposed to DSS, but did receive daily SC injections of vehicle solution on day 4 to 9. Animal survival was recorded daily, and the experiment was concluded on day 10. Note that the disease control and the 0.2 mg/kg FGF-20 groups yielded identical results and are both represented by red squares.

protein products exert a protective effect in intestinal inflammation.^{23,24} The result of this experiment indicates that FGF-20 (100 ng/mL) stimulates the expression of both of these genes in HT-29 and Caco-2 human colonic epithelial cancer cells (Figure 10). Peak up-regulation was seen following exposure of cells to FGF-20 for 1–3 hours (COX-2) or 3–6 hours (ITF). An increase in COX-2 protein expression after exposure of HT-29 and Caco-2 cells to FGF-20 for 3 hours was evident via Western blot analysis (data not shown).

Because prostaglandins have been implicated in mucosal healing²³ and PGE₂ production is stimulated by COX-2, we examined the effect of FGF-20 on PGE₂ levels in HT-29 and Caco-2 cells. The result of this experiment indicates that FGF-20 significantly enhances the levels of PGE₂ in a dose-dependent fashion in both of these cell lines. The highest FGF-20 dose examined (100

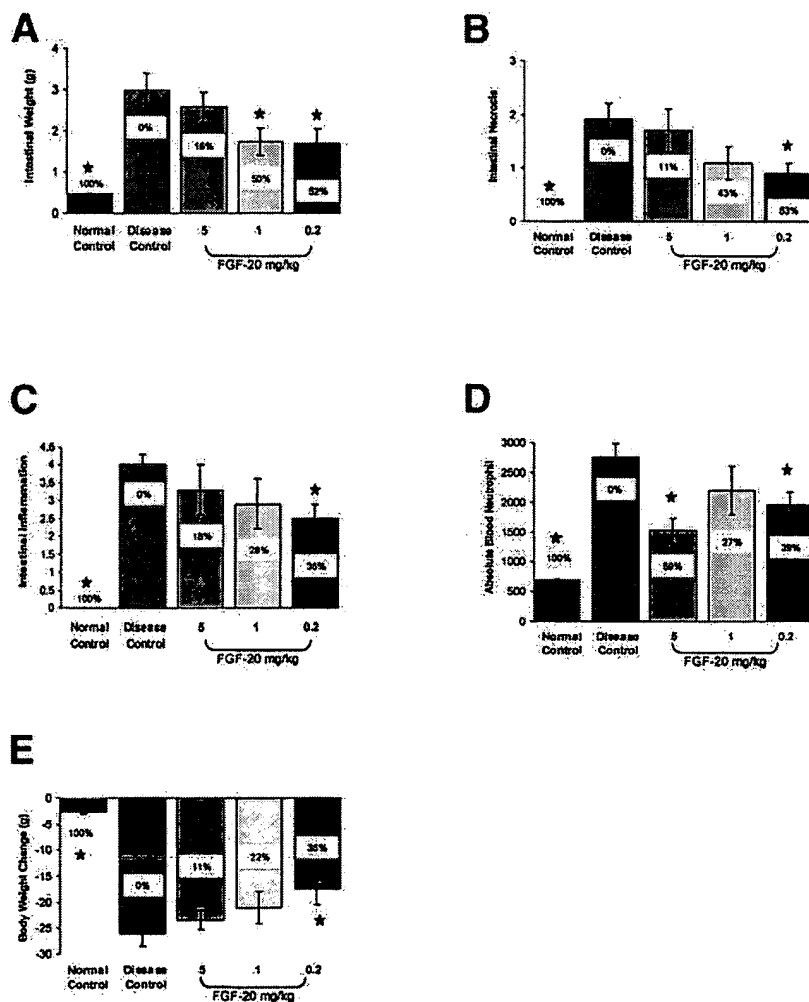


Figure 6. Effects of FGF-20 on indomethacin-induced intestinal ulcerations. To induce intestinal lesions, female Lewis rats were given indomethacin (7.5 mg/kg) SC for 2 days (day 0 to 1). Disease control animals ($n = 8$) received daily IV injections of vehicle solution on day -1 to day 4. FGF-20-treated groups ($n = 8$ animals/group) received daily IV injections of FGF-20 at the indicated concentrations on day -1 to day 4. Normal control animals ($n = 4$) did not receive indomethacin, but did receive daily IV injections of vehicle solution on day -1 to 4. Animals were sacrificed on day 5, and a 10 cm section of the distal jejunum in the area at risk for lesions was (A) weighed and histologically examined and scored for level of (B) necrosis and (C) inflammation. (D) The absolute neutrophil counts obtained from blood harvested at necropsy. (E) The change in total body weight from day 0 to 5. Results are reported as mean \pm standard error, and the percentage changes indicated are relative to the disease control group. Changes that are statistically different from the disease control group ($P < 0.05$ by the 2-tailed Student *t* test) are indicated with a star.

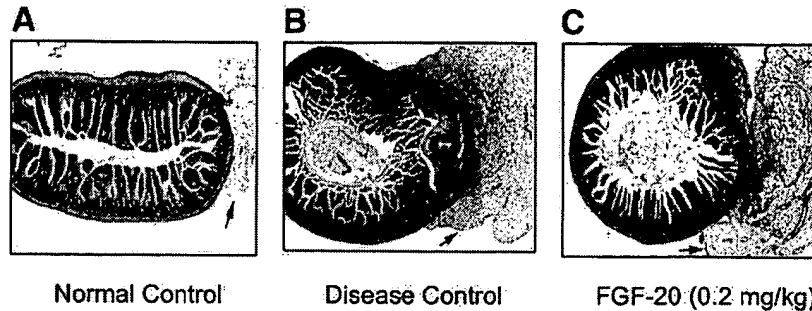


Figure 7. Effects of FGF-20 on indomethacin-induced intestinal ulcerations: histopathology. Representative sections of the distal jejunum from the area at risk were collected at necropsy, preserved in formalin, stained with H&E, magnified 25 \times , and photographed. The groups are as described in the legend to Figure 6. Arrows indicate attached mesentery. Note that FGF-20 (0.2 mg/kg) inhibits the necrosis associated with indomethacin treatment.

ng/mL) stimulated PGE₂ production to a similar degree as that of the positive control (10% FBS).

Discussion

FGF-20 is a novel growth factor that exhibits proliferative activity on fibroblasts and epithelial cells.¹⁹ Because both of these cell types play important roles in

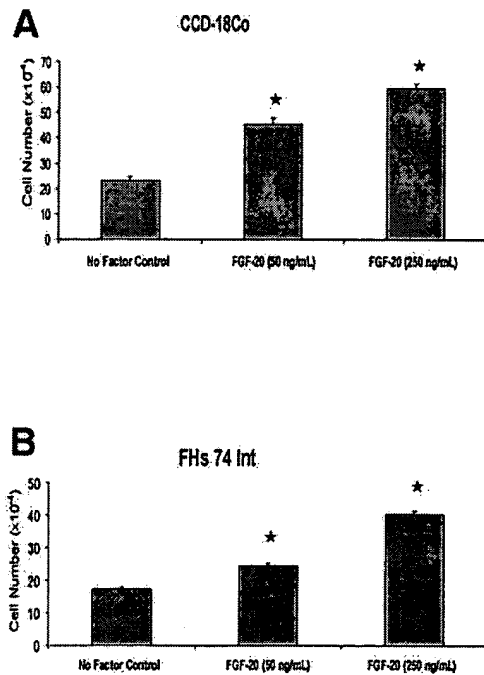


Figure 8. Effects of FGF-20 on the growth of human intestinal cells in vitro. CCD-18Co human colonic fibroblasts (A) and FHS 74 Int human intestinal epithelial cells (B) were cultured without or with the indicated concentrations of FGF-20 for 6 days and then counted. Data points represent the mean of duplicate wells \pm standard deviation. Changes that are statistically different from control cells receiving no factor ($P < 0.05$ by the 2-tailed Student *t* test) are indicated with a star. The experiment was performed 2 times with similar results.

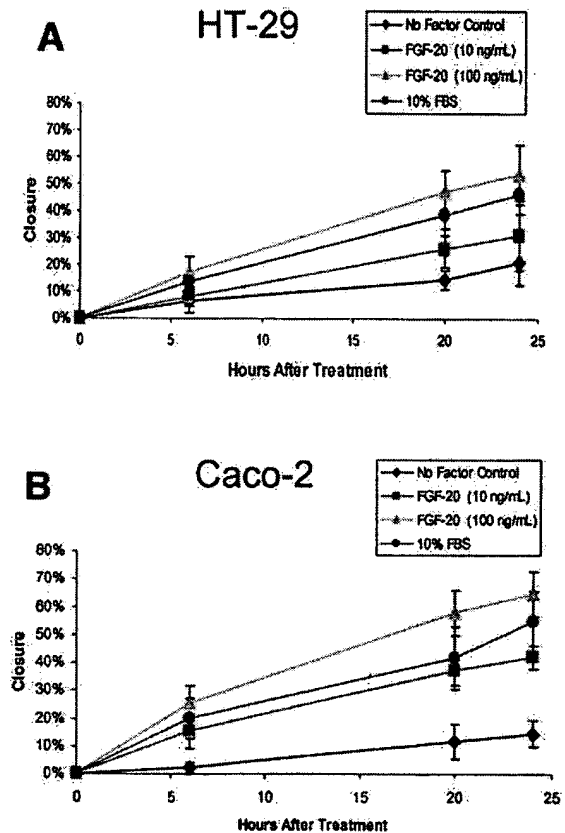


Figure 9. Effects of FGF-20 on the restitution of human colonic cells in vitro. Monolayers of "wounded" HT-29 (A) and Caco-2 (B) human colonic epithelial cancer cells were cultured without or with the indicated concentrations of FGF-20, or 10% FBS as a positive control, and wound width was measured after 0, 6, 20, and 24 hours. Each data point represents the mean of 12 wounds \pm standard deviation, and results are reported as the percentage of closure relative to values obtained at time 0. All FGF-20 data points, with the exception of the treatment of HT-29 with 10 ng/mL FGF-20 for 6 hours, are statistically different ($P < 0.05$ by the 2-tailed Student *t* test) from control cells receiving no factor. The experiment was performed 3 times with similar results.

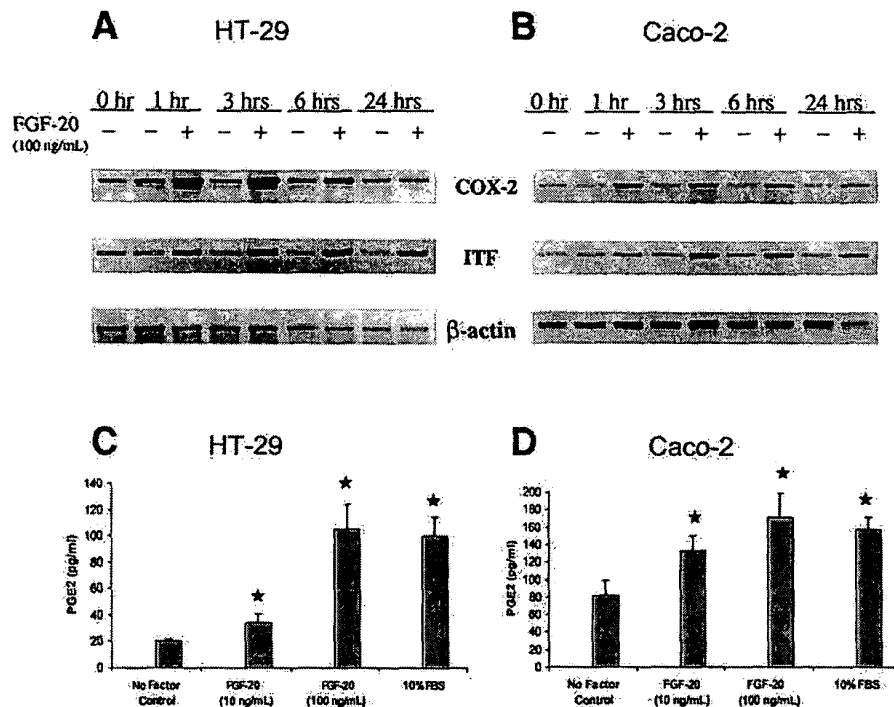


Figure 10. Effects of FGF-20 on the expression of COX-2 and ITF mRNA and on PGE₂ levels in human colonic cells in vitro. The expression of COX-2, ITF, and β -actin RNA transcripts in HT-29 (A) and Caco-2 (B) human epithelial cancer cells cultured without or with FGF-20 (100 ng/mL) for the indicated time periods was determined by RT-PCR. PGE₂ levels in the media of HT-29 (C) and Caco-2 (D) cells cultured for 24 hours without or with the indicated concentrations of FGF-20, or 10% FBS as a positive control, were determined by ELISA. Each data point represents the mean of 9 wells \pm standard deviation. Changes that are statistically different from control cells receiving no factor ($P < 0.05$ by the 2-tailed Student *t* test) are indicated with a star. The experiment was performed 3 times with similar results.

tissue repair,⁴ we sought to examine the effect of FGF-20 in a tissue repair model. To this end, we initiated a series of experiments to explore the effect of FGF-20 in animal models of IBD, a disease in which the integrity of the intestinal epithelium is compromised¹⁻³ and for which additional therapies are needed. Further rationale for assessing the activity of FGF-20 in IBD animal models originates from the finding that FGF-20 interacts strongly with various FGFRs, including FGFR2b, FGFR2c, and FGFR3c,¹⁹ at least 1 of which is present on intestinal epithelium,^{7,8} and from previous studies implicating FGFs in intestinal epithelial repair.^{7,13-18,25}

The present findings demonstrate that FGF-20 is active in 2 independent rodent IBD models: DSS treatment of mice to induce an ulcerative colitis-like syndrome, and indomethacin treatment of rats to induce a Crohn's-like disease consisting of inflammation and ulceration of the small bowel. The decision to use these 2 models was based on the fact that each model affects a different region of the gastrointestinal tract and thus may represent different human disease counterparts. Moreover, the pathologies associated with these models are highly reproducible.²²

In the DSS model, a significant beneficial effect of FGF-20, as indicated by various experimental parameters, was evident when this growth factor was administered concomitantly with DSS. This effect was seen regardless of whether FGF-20 was administered through the IP or the SC route. In addition, a beneficial effect of FGF-20 on animal survival was found when FGF-20 was administered therapeutically after 4 days of DSS exposure. The protective effects of FGF-20 in the DSS model occurred in a dose-dependent fashion, with maximum protection observed at the highest concentration examined (5 mg/kg). FGF-20-administered IV also proved active in the rat indomethacin model, in which an inverse dose-response was observed. Bimodal dose-response curves have been reported for other biological molecules,^{13,26,27} and it is possible that the activity of FGF-20 in the DSS and indomethacin models fall on opposite sides of the response curve.

The in vitro studies presented herein suggest multiple mechanisms for explaining how FGF-20 stimulates intestinal healing. For example, FGF-20 may be enhancing mucosal repair, a hypothesis supported by

the finding that this factor increases the restitution of colonic epithelial cells and the growth of colonic fibroblasts and intestinal epithelial cells. Because ITF has been shown to accelerate epithelial restitution^{24,28} and FGF-20 increases ITF expression in colonic epithelia, it is also possible that ITF mediates at least some of the mucosal repair induced by FGF-20. Additional properties of ITF that may contribute to the support of the mucosal barrier include its ability to increase mucus viscosity²⁹ and prevent epithelial cell apoptosis.³⁰ The data further suggest that FGF-20 may enhance mucosal healing by stimulating a COX-2-mediated increase in PGE₂, a molecule which has been shown to stimulate the healing process.^{23,31} The net effect of accelerated epithelial restitution, restoration of an intact epithelial layer, and improved mucosal barrier function after FGF-20 treatment is decreased mucosal permeability and reduced inflammation caused by decreased uptake of inflammation-inducing substances, including bacterial antigens, cell wall polymers, and chemotactic peptides.¹ Moreover, PGE₂ (generated by COX-2 by way of FGF-20) may reduce inflammation by inhibiting inflammatory cell activation.³²

There are currently no marketed drugs for IBD that stimulate intestinal repair, although at least 2 FGF family members (FGF-7 and FGF-10, also known as KGF-1 and KGF-2, respectively), have shown activity in animal models of IBD.^{13,17} Because of their narrow receptor specificity, FGF-7 and FGF-10 efficiently activate some epithelial cells, but not fibroblasts.³³ However, FGF-20 interacts with a variety of FGF receptors¹⁹ and thus is capable of activating fibroblasts as well as epithelial cells. Evidence suggests that fibroblasts play an important role in the repair of epithelium,⁴ and thus FGF-20 may be well suited for the treatment of this disease.

A biological agent for the treatment of IBD, an antibody to TNF- α , has proven useful in the treatment of moderate to severe Crohn's disease.³⁴ However, use of this product increases the risk of infection.³⁴ In contrast, FGF-20 represents a new class of biological agent that may prove effective in the treatment of IBD by stimulating intestinal repair without increasing the risk of infection.

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Dr. Yang owns stocks in CuraGen and is no longer affiliated with the company.

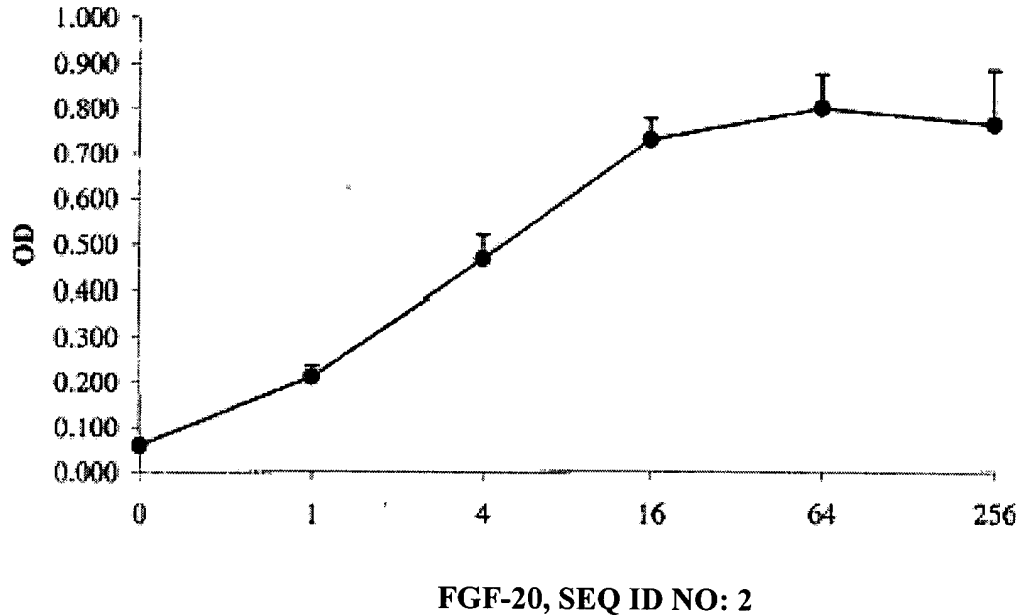
The authors thank Jane Mayotte and Kelly McQueeney for their valuable assistance.

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Exhibit 2

Proliferation and Neutralization Assays

FIGURE 1: Dose response of Cellular Proliferation to FGF-20, SEQ ID NO: 2



NIH 3T3 cells were plated in 96-well dishes, cultured for 5 days, and washed with DMEM + 0.1% BSA. The cells were then treated with CG53135-05 diluted in DMEM + 0.1% BSA. DNA synthesis was measured using BrdU incorporation assay, and the OD was measured at 450 nm. Data points represent the average OD of triplicate samples \pm standard deviation

Proliferation Assay (Figure 1):

The effect of FGF-20 protein (SEQ ID NO:2) on proliferation of NIH 3T# cells was determined, and a dose response curve was generated. The protein of the instant specification induced DNA synthesis in NIH 3T3 cells in a concentration-dependant manner (FIG. 1). The EC_{75} for the protein was 10ng/ml and thus this concentration of the protein was utilized in subsequent neutralization experiments detailed in FIG. 2.

FIGURE 2: ClustalW Protein Sequence Alignment Analysis

Sequences analyzed:

1. rat-FGF-20 (rat FGF-20)
2. ms-FGF-20 (mouse FGF-20)
3. h-FGF-20 (human FGF-20)
4. FGF-CX (SEQ ID NO: 1)

Clustal Details:

CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein

Sequence format is Pearson

Sequence 1: rat-FGF-20 212 aa

Sequence 2: ms-FGF-20 212 aa

Sequence 3: h-FGF-20 211 aa

Sequence 4: FGF-CX 211 aa

Multiple Alignment:

```
h-FGF-20  MAPLAEVGGFLGGLEGLGQQVGSFLLPPAGERPPLLGERRSAAERSARGGPGAAQLAHL
FGF-CX     MAPLAEVGGFLGGLEGLGQQVGSFLLPPAGERPPLLGERRSAAERSARGGPGAAQLAHL
rat-FGF-20 MAPLTEVGAFLLGGLEGLGQQVGSFLLPPAGERPPLLGERRGALERGARGGPGSVELAHL
ms-FGF-20  MAPLTEVGAFLLGGLEGLGQQVGSFLLPPAGERPPLLGERRGALERGARGGPGSVELAHL

h-FGF-20  HGILRRRQLYCRTGFHLQILPDGSVQGTRQDHSLEFGILEFISVAVGLVSIrgvdsGLYLG
FGF-CX     HGILRRRQLYCRTGFHLQILPDGSVQGTRQDHSLEFGILEFISVAVGLVSIrgvdsGLYLG
rat-FGF-20 HGILRRRQLYCRTGFHLQILPDGSVQGTRQDHSLEFGILEFISVAVGLVSIrgvdsGLYLG
ms-FGF-20  HGILRRRQLYCRTGFHLQILPDGTVQGTRQDHSLEFGILEFISVAVGLVSIrgvdsGLYLG

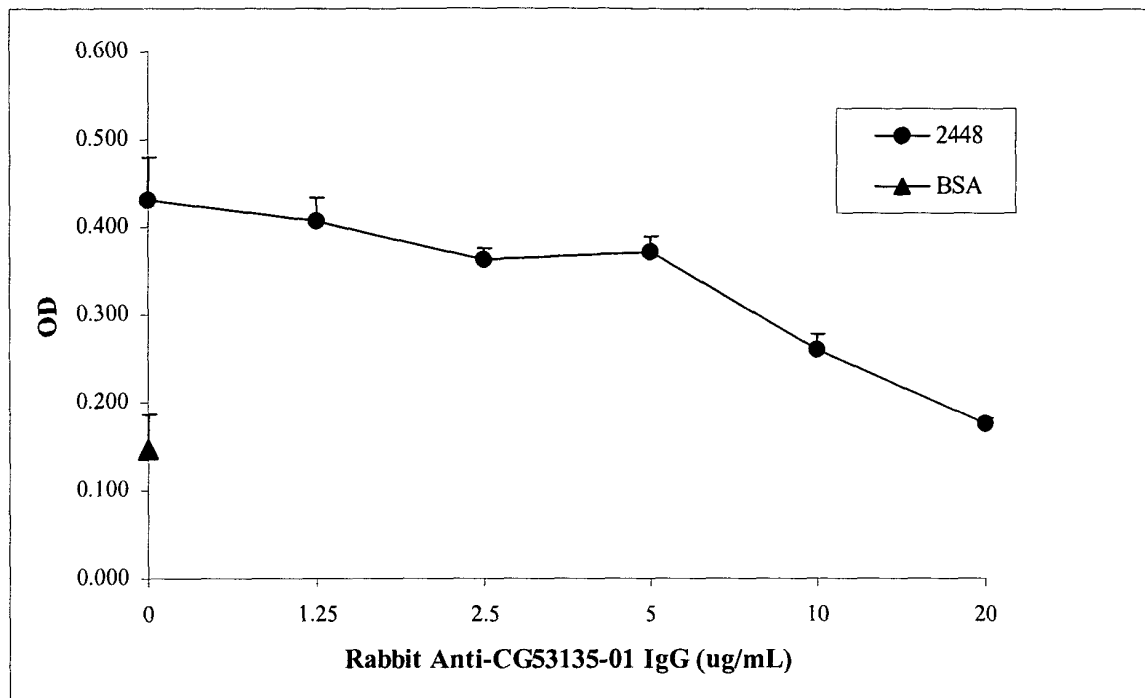
h-FGF-20  MNDKGELYGSEKLTSECFREQFEENWYNTYSSNIYKHGDTGRRYFVALNKDGTPrdGAR
FGF-CX     MNDKGELYGSEKLTSECFREQFEENWYNTYSSNIYKHGDTGRRYFVALNKDGTPrdGAR
rat-FGF-20 MNDKGELYGSEKLTSECFREQFEENWYNTYSSNIYKHGDTGRRYFVALNKDGTPrdGAR
ms-FGF-20  MNDKGELYGSEKLTSECFREQFEENWYNTYSSNIYKHGNTGRRYFVALNKDGTPrdGAR

h-FGF-20  SKRHQKFTHFLPRPVDPERVPELYKDLLMYT-
FGF-CX     SKRHQKFTHFLPRPVDPERVPELYKDLLMYT-
rat-FGF-20 SKRHQKFTHFLPRPVDPERVPELYKDLLMYTG
ms-FGF-20  SKRRQKFTHFLPRPVDPERVPELYKDLLMYTG
```

FIGURE 3: Neutralization Assay

Neutralization of CG53135-05-stimulated Cellular Proliferation by Rabbit Polyclonal Anti-CG53135-01 IgG

Rabbit polyclonal anti-FGF 20 IgG purified from rabbit 2448 antiserum neutralized FGF 20 (SEQ ID NO: 2)-stimulated proliferation of NIH 3T3 cells in a dose-dependent manner. At 20 $\mu\text{g/ml}$ concentration, the inhibition of cellular proliferation was 90% with 2448 antiserum.



NIH 3T3 cells were plated in 96-well dishes, cultured for 5 days, and washed with DMEM + 0.1% BSA. The cells were then treated with CG53135-05 (10 ng/mL) containing serial dilutions of rabbit polyclonal anti-CG53135-01 IgG purified from rabbit 2448 antiserum (2448), or were treated with 0.1% BSA alone (BSA). DNA synthesis was measured using a BrdU incorporation assay, and the OD was measured at 450 nm. Data points represent the average OD of triplicate samples \pm standard deviation.

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Exhibit 3

FDA's approval of the IND

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CuraGen Corporation (ticker: CRGN, exchange: NASDAQ) News Release - 4-Mar-2003

CuraGen Receives FDA Approval to Initiate Clinical Trials

Potential Oral Mucositis Treatment Marks Successful Transition into Drug Development

NEW HAVEN, Conn., Mar 4, 2003 /PRNewswire-FirstCall via COMTEX/ --CuraGen Corporation (Nasdaq: CRGN), a genomics-based pharmaceutical company, today announced that the U.S. Food and Drug Administration (FDA) has approved its Investigational New Drug (IND) application to initiate clinical trials for CG53135, a potential protein therapeutic being investigated as a treatment for oral mucositis. Oral mucositis is a side effect of chemotherapy and radiotherapy that results in the degradation of mucosal tissue that can range from redness and irritation to severe ulcerations of the mouth and throat. CuraGen now plans to proceed with a multi-center Phase I clinical trial to evaluate safety and pharmacokinetics in patients with cancer who are at risk for mucositis following chemotherapy.

Mucositis is a debilitating complication of cancer chemotherapy or radiotherapy that affects the mucosal tissue, which acts as a protective lining within the digestive track, including the mouth and throat. Symptoms range from pain and discomfort to severe ulcerations that limit a patient's ability to ingest nutrients. Mucositis can result in a suppressed immune system that can reduce a patient's ability to tolerate further cancer therapy. Delayed treatment can lessen the effectiveness of the chemotherapy or radiotherapy, adversely impacting the value of the patient's overall treatment regimen.

"CG53135 is a novel protein discovered through the application of CuraGen's functional genomic technologies. In preclinical studies, this potential protein therapeutic reduced tissue inflammation and degeneration, and minimized the severity and extent of mucosal tissue damage. Mucositis is a significant unmet medical need, and we are pleased to have the opportunity to advance this promising molecule into human clinical trials," stated Timothy M. Shannon, M.D., Senior Vice President of R&D and Chief Medical Officer of CuraGen Corporation.

"Through the filing of this IND, CuraGen has become one of the first genomics companies to successfully transition from a target discovery company into a genomics-based pharmaceutical company. This molecule represents the first of many promising candidates that we believe will emerge from our portfolio of discovery and preclinical stage projects. We are pleased with the progress of this potential therapeutic and look forward to additional future successes," stated Jonathan M. Rothberg, Ph.D., Founder, Chairman, and CEO of CuraGen Corporation.

About CuraGen

CuraGen Corporation (NASDAQ: CRGN) is a genomics-based pharmaceutical company. CuraGen's integrated, functional genomic technologies and Internet-based bioinformatic systems are designed to generate comprehensive information about genes, human genetic variations, gene

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pathways. The Company is applying its industrialized genomic technologies, informatics, and validation technologies to develop protein, antibody, and small molecule therapeutics to treat obesity and diabetes, cancer, inflammatory diseases, and central nervous system (CNS) disorders. CuraGen is headquartered in New Haven, CT and additional information is available at www.curagen.com.

This press release may contain forward-looking statements including statements about CG53135's demonstrated ability to reduce tissue inflammation and degeneration, and minimize the severity and extent of mucosal tissue damage in preclinical studies, as well as representing the first of many promising candidates that we believe will emerge from our portfolio of discovery and preclinical stage projects. Such statements are based on management's current expectations and are subject to a number of factors and uncertainties that could cause actual results to differ materially from those described in the forward-looking statements. CuraGen cautions investors that there can be no assurance that actual results or business conditions will not differ materially from those projected or suggested in such forward-looking statements as a result of various factors, including, but not limited to, the following: CuraGen's expectation that it will incur operating losses in the near future, the early stage of development of CuraGen's products and technologies, uncertainties related to preclinical and clinical testing and trials, uncertainties and adverse results relating to CuraGen's ability to obtain regulatory approval for its products in development, uncertainties surrounding the availability of additional funding, CuraGen's reliance on research collaborations and strategic alliances, the actions of competitors, the development of competing technologies, CuraGen's ability to protect its patents and proprietary rights, patent infringement actions and uncertainties relating to commercialization rights. Please refer to our Annual Report on Form 10-K for the fiscal year ended December 31, 2001 for a description of these risks. We disclaim any intention or obligation to update or revise any forward-looking statements, whether as a result of new information, future events, or otherwise.

SOURCE CuraGen Corporation

<http://www.curagen.com/ir/FShareholder.htm>

Exhibit 4

PROSITE software Domain Analysis

PROSITE - Protein Domain Matches for Gene ID: FGF20X

Pattern-ID: PKC_PHOSPHO_SITE PS00005 (Interpro) PDOC00005

Pattern-DE: Protein kinase C phosphorylation site

Pattern: [ST].[RK]

47	SAR
109	SIR
130	SEK
161	TGR
174	TPR
181	SKR

Pattern-ID: CK2_PHOSPHO_SITE PS00006 (Interpro) PDOC00006

Pattern-DE: Casein kinase II phosphorylation site

Pattern: [ST].{2}[DE]

42	SAAE
88	TRQD
174	TPRD

Pattern-ID: TYR_PHOSPHO_SITE PS00007 (Interpro) PDOC00007

Pattern-DE: Tyrosine kinase phosphorylation site

Pattern: [RK].{2,3}[DE].{2,3}Y

111	RGVDSGLY
140	REQFEENWY

Pattern-ID: MYRISTYL PS00008 (Interpro) PDOC00008

Pattern-DE: N-myristoylation site

Pattern: G[^EDRKHPFYW].{2}[STAGCN][^P]

8	GGFLGG
18	GQQVGS
50	GGPGAA
83	GSVQGT
112	GVDSGL

Pattern-ID: AMIDATION PS00009 (Interpro) PDOC00009

Pattern-DE: Amidation site

Pattern: .G[RK][RK]

161	TGRR
-----	------

Pattern-ID: HBGF_FGF PS00247 (Interpro) PDOC00220

Pattern-DE: HBGF/FGF family signature

Pattern: G.[LIM].[STAGP].{6,7}[DE]C.[FLM].E.{6}Y

125	GELYGSEKLTSECIFREQFEENWY
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